Supplemental Information

Fig. S1. Crystallographic analysis. (a) The asymmetric unit harbors three copies of the mNup98^{APD}•yNup82^{NTD}•yNup159^{T} heterotrimer that interact with each other in a head-to-tail fashion and are related by an approximate 3-fold axis. A 90°-rotated view is shown on the right. (b) Representative final 2|fo|-|fc| electron density maps, rendered at 1.0 σ, illustrating the three interfaces in the mNup98^{APD}•yNup82^{NTD}•yNup159^{T} heterotrimer.

Fig. S2. Cartoon and schematic representations of the three interfaces in the heterotrimer. (a) FGL-loop interaction. (b) K-loop interaction. (c) yNup159^{T} interaction. Residues are colored according to Figure 4. Van der Waals contacts and polar interactions are represented by dashed and solid black lines, respectively.

Fig. S3. Multi-species sequence alignment for yNup82^{NTD} homologs. The numbering below the alignment is relative to *S. cerevisiae* Nup82. The overall sequence conservation at each position is shaded in a color gradient from yellow (40 % similarity) to dark red (100 % identity) using the Blosum62 weighting algorithm. The secondary structure is indicated above the sequence as blue boxes (α helices), green arrows (β strands), gray lines (coil regions), and gray dots (disordered residues). Residues involved in the interaction with mNup98^{APD} and yNup159^{T} are indicated by yellow and orange dots, respectively. The positions of the DFY, LILLF, and ΔFGL loop mutations are labeled below the dots.

Fig. S4. Multi-species sequence alignment for mNup98^{APD} and yNup159^{T} homologs. (A) The numbering below the alignment is relative to *M. musculus* Nup98. The positions of the invariant K loop K831 and catalytic S881 are indicated by an asterisk and an arrowhead, respectively. The three bottom sequences refer to the three GLFG nucleoporins in *S. cerevisiae*, including the Nup98 homolog yNup145N. (B) The numbering below the alignment is relative to *S. cerevisiae* Nup159. Residues involved in the interaction with yNup82^{NTD} are indicated by magenta dots.

The labeling of the secondary structure elements and conservation coloring scheme of the alignment are according to Figure S3.

Fig. S5. Quantitation of the size exclusion chromatography interaction analysis. Three representative interactions analysis showing wild-type binding (+), intermediate binding (+/-), and no binding (-).

Table S1. Bacterial and yeast expression constructs.

[a] All yNup82^{NTD} constructs used for bacterial expression carry a C396S mutation

[b] Constructs used for crystallization of the mNup98^{APD}•yNup82^{NTD}•yNup159^{T} heterotrimer

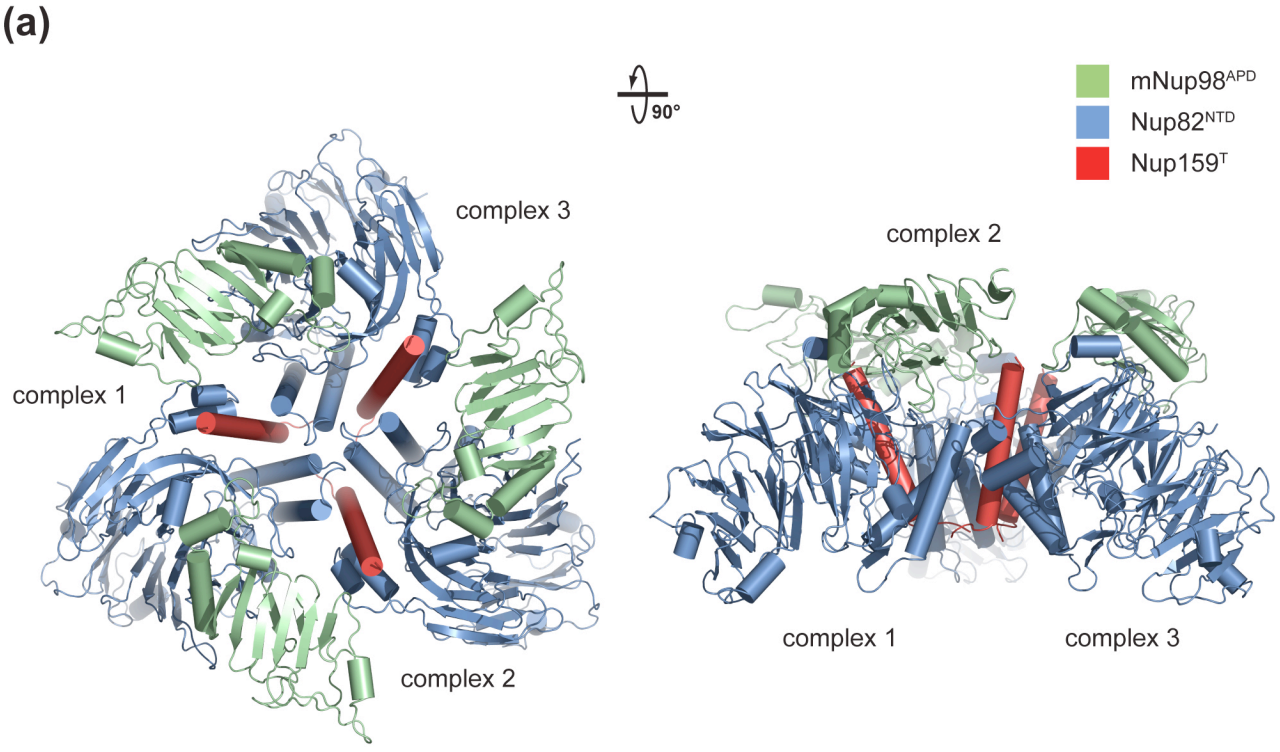

Figure S1, Stuwe et al., 2012

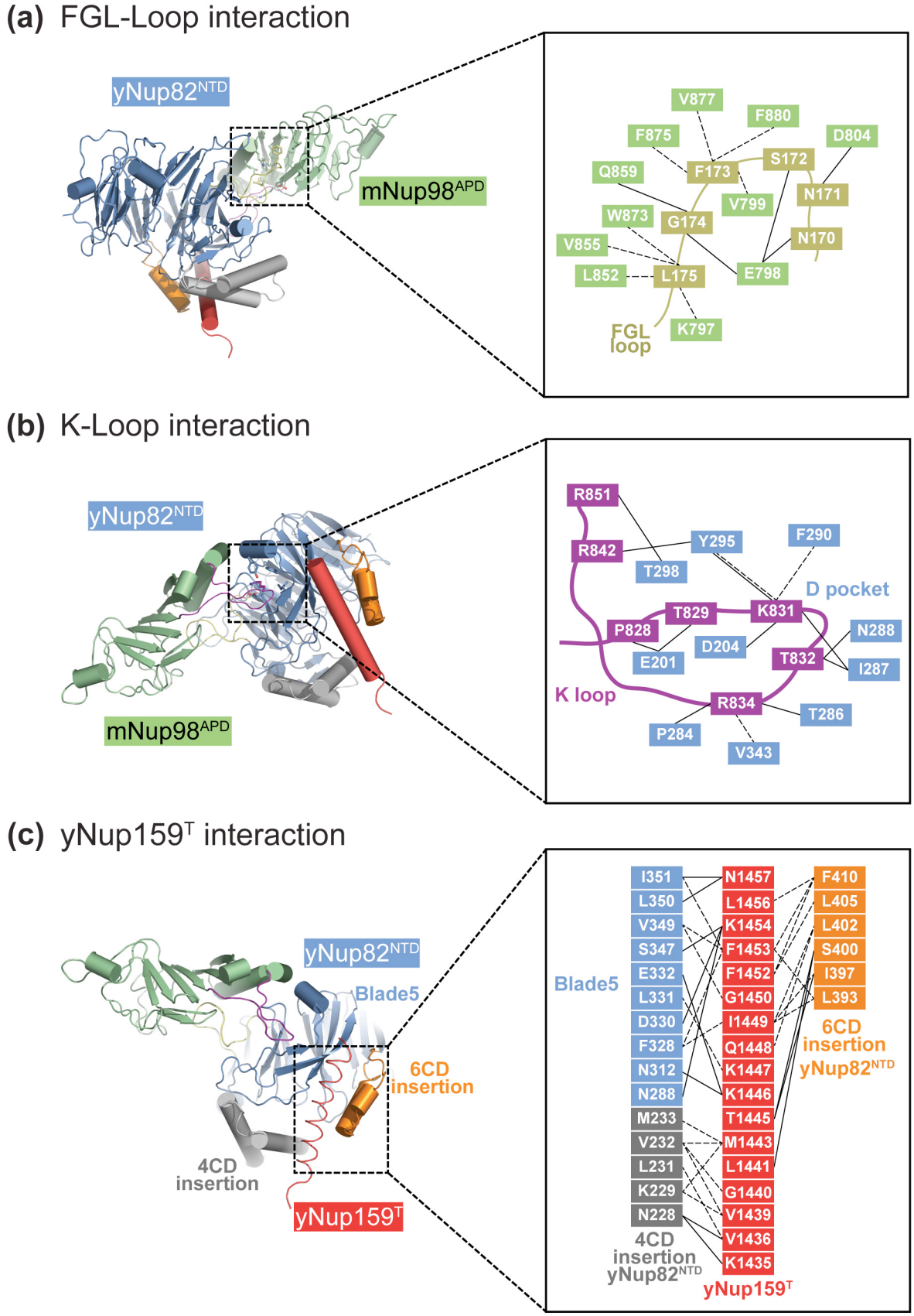

Figure S2, Stuwe et al., 2012

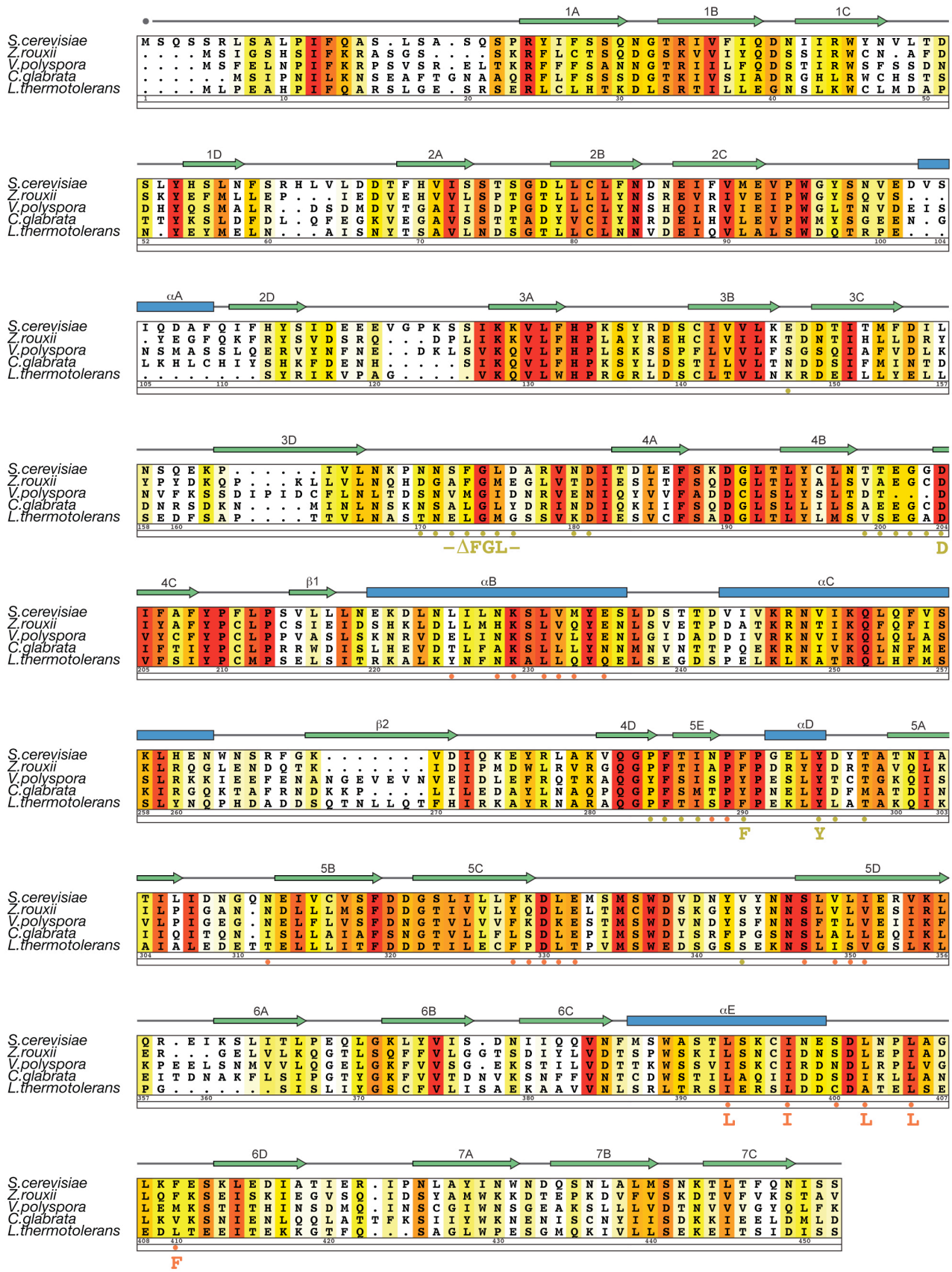

Figure S3, Stuwe et al., 2012

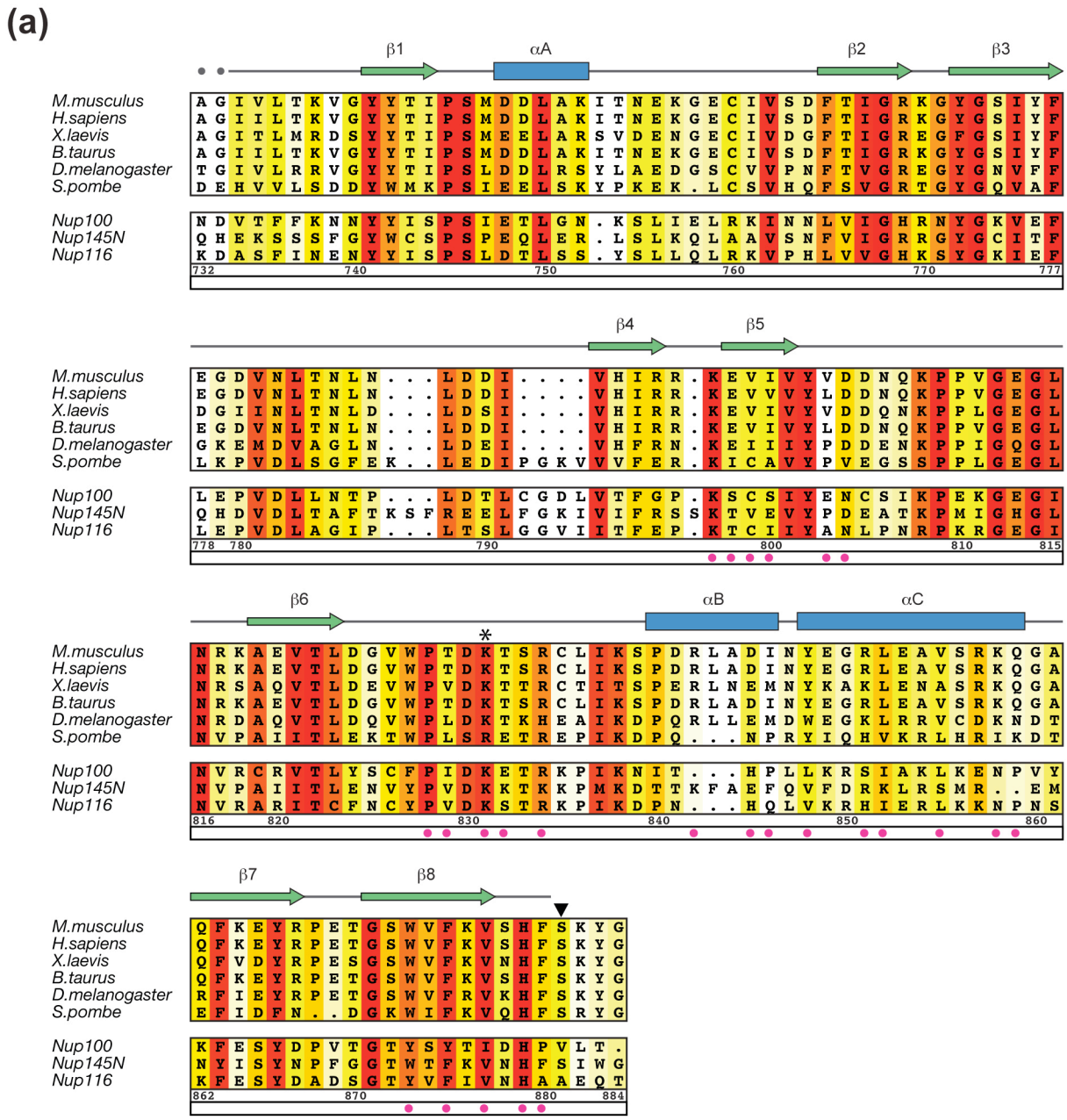

Figure S4, Stuwe et al., 2012

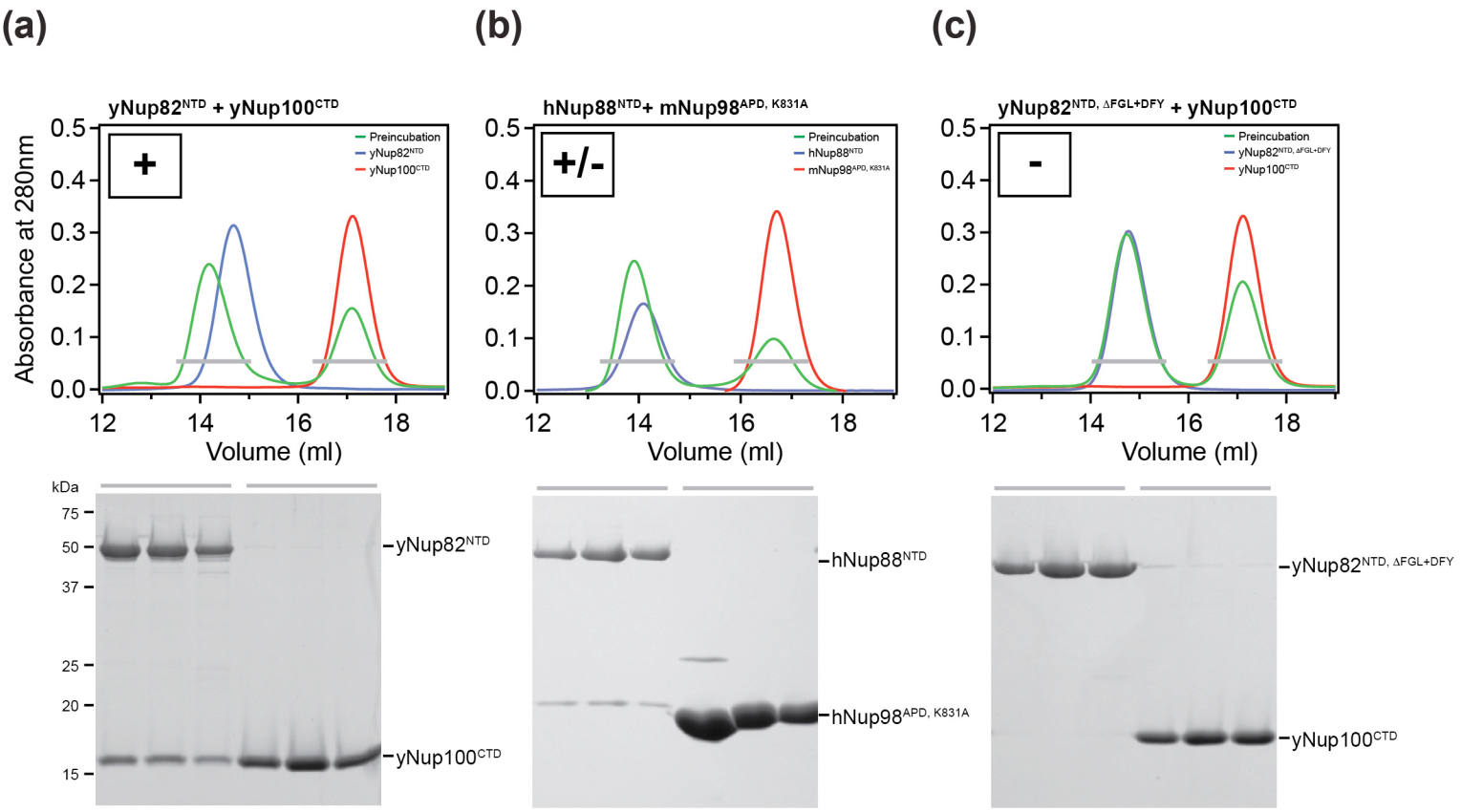

Figure S5, Stuwe et al., 2012

Table S1. Expression constructs

Bacterial expression constructs[a]

Protein	Residues (Mutations)	Expression vector	Restriction sites 5', 3'	N-terminal overhang
yNup82^{NTD}	1-452	pET28a-PreS	NdeI, XhoI	GPH
yNup82$^{NTD, \Delta FGL}$	1-452 (ΔFGL: Δ172-176)	pET28a-PreS	NdeI, XhoI	GPH
yNup82$^{NTD, DFY}$	1-452 (D204A, F290A, Y295A)	pET28a-PreS	NdeI, XhoI	GPH
yNup82$^{NTD, LILLF}$	1-452 (L393A, I397A, L402A, L405A, F410A)	pET28a-PreS	NdeI, XhoI	GPH
yNup82$^{NTD, \Delta FGL+DFY}$	1-452 (ΔFGL + DFY)	pET28a-PreS	NdeI, XhoI	GPH
yNup82$^{NTD, \Delta FGL+DFY+LILLF}$	1-452 (ΔFGL + DFY + LILLF)	pET28a-PreS	NdeI, XhoI	GPH
mNup98^{APD}	732-880	pET28a-PreS[b]	NdeI, XhoI	GPHM
mNup98^{APD}	732-880 (K831A)	pET28a-PreS	NdeI, NotI	GPHM
hNup98^{APD}	715-920	pET28a-PreS[b]	NdeI, XhoI	GPHM
yNup100^{CTD}	814-960	pET28a-PreS	NheI, XhoI	GPHM
yNup116^{CTD}	967-1113	pET28a-PreS	NdeI, NotI	GPHM
yNup145N^{APD}	458-605	pET28a-PreS	NheI, XhoI	GPHM
yNup159^{T}	1425-1460	pET28b-SUMO	BamHI, HindIII	His_6-SUMO
yNup159^{T}	1425-1460	pETDuet-1[b]	NcoI, NotI	GPHM
yNup82^{NTD}	1-452		NdeI, XhoI	none
yNup145C	1-711	pETDuet-1	BamHI, NotI	His_6-SUMO
ySec13	1-297	pET24b	NdeI, XhoI	none

Yeast Expression Constructs

Protein	Residues (Mutations)	Shuffle Vector	Restriction Sites 5', 3'	Selection
Nup82^{WT}	1-713	pRS416-mCherry	NotI, SacII	Ura
Nup82^{WT}	1-713	pRS315-GFP	XhoI, ApaI	Leu
Nup82$^{\Delta NTD}$	453-713	pRS315-GFP[b]	XhoI, ApaI	Leu
Nup82$^{\Delta FGL+DFY}$	1-713 (ΔFGL: Δ172-176; DFY: D204A, F290A, Y295A)	pRS315-GFP	XhoI, ApaI	Leu
Nup82^{LILLF}	1-713 (LILLF: L393A, I397A, L402A, L405A, F410A)	pRS315-GFP[b]	XhoI, ApaI	Leu
Nup82$^{\Delta FGL+DFY+LILLF}$	1-713 (ΔFGL; DFY; LILLF)	pRS315-GFP	XhoI, ApaI	Leu

[a] All yNup82^{NTD} constructs used for bacterial expression carry a C396S mutation

[b] Constructs used for crystallization of the mNup98^{APD}•yNup82^{NTD}•yNup159^{T} heterotrimer